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MHC Class II Expression in Double Mutant Mice Lacking Invariant Chain and DM Functions

George Kenty,* W. David Martin,2† Luc Van Kaer,3† and Elizabeth K. Bikoff4*

Invariant (Ii) chain and DM functions are required at distinct stages during class II maturation to promote occupancy by diverse peptide ligands. The class II molecules expressed by mutant mouse strains lacking Ii chain or DM activities display discrete structural and functional abnormalities. The present report describes the cellular and biochemical characteristics of Ii−DM− doubly deficient mice. As for Ii chain mutants, their mature αββ dimers similarly exhibit reduced mobilities in SDS-PAGE, and in functional assays these molecules behave as if empty or occupied by an easily displaced peptide. Additionally, the present experiments demonstrate that the production of floppy αββ dimers is TAP independent. In comparison with Ii chain mutants, Ii−DM− doubly deficient cell populations exhibit increased peptide binding activities and consistently greater presentation abilities in T cell stimulation assays. These functional differences appear to reflect higher class II surface expression associated with their increased representation of B lymphocytes. We also observe defective B cell maturation in mice lacking Ii chain or DM expression, and interestingly, B cell development appears more severely compromised in Ii−DM− double mutants. These mutant mice lacking both Ii chain and DM activities should prove useful for analyzing nonconventional class II Ag presentation under normal physiological conditions in the intact animal.

Considerable progress has been made over recent years toward understanding the complex cellular and biochemical events necessary for guiding MHC class II peptide acquisition. Important contributions are made by both the invariant (Ii) chain and DM to facilitate surface expression of diverse peptide ligands, but these activities are required at distinct stages of class II maturation. Ii chain confers posttranslational folding and assembly on β subunits in the endoplasmic reticulum (ER). In the exceptional case of αββ molecules, the Ii chain is essential for production or maintenance of αβ dimers (3). This early class II association with the Ii chain probably prevents irreversible misfolding or aggregation of the subunits and protects the empty peptide groove from association with molecular chaperones such as BiP and calnexin that are responsible for ER quality control (4–8). The Ii chain facilitates export of correctly folded αβ dimers past the Golgi complex (9, 10) and directs their delivery to the endocytic compartment(s) (11, 12). Selective Ii chain degradation subsequently permits occupancy of the class II peptide groove (13).

The nonconventional class II product DM, originally described as a facilitator of Ag presentation in mutant human cell lines, acts later inside the endocytic compartment(s) to cause dissociation of a relatively short proteolytic product of the Ii chain corresponding to the so-called CLIP region, in exchange for tightly bound peptide ligand(s) (14, 15). Recent studies demonstrate the DM also associates with empty class II molecules (16–18) and acting in this manner may function as a peptide editor, serving to increase the overall affinities of peptide/class II complexes (19, 20). DM contains its own endosomal targeting signal(s) located in the β chain cytoplasmic tail (21, 22), but there is also evidence suggesting that DM transport is mediated via an association with the Ii chain (21). In contrast to αβ-Ii complexes formed early during biosynthesis, far less is known about transient associations among class II αβ dimers, Ii chain degradation products, and DM molecules during peptide acquisition in the endocytic compartment(s).

The specific defects described for Ii chain (23–25) and DM (26–28) mutant mouse strains created using embryonic stem cell technology partially overlap. Both these mutations disrupt class II maturation, Ag presentation, and CD4+ T cell development. However, DM mutant spleen cells efficiently express surface αββ/CLIP complexes at levels equivalent to those in wild-type αββ molecules (26–28). In contrast, the loss of Ii chain function leads to markedly reduced αββ surface expression, largely due to decreased rates of export (23–25). Thus in the absence of Ii chain, the vast majority of class II αβ dimers fail to acquire endoglycosidase H-resistant glycans and are rapidly degraded. The few mature αββ dimers produced by Ii chain mutants exhibit reduced mobilities in SDS gels, and in functional assays these molecules behave as if empty or occupied by an easily displaced peptide. The structural basis for the exceptional abilities of these floppy αββ dimers to escape ER quality control has yet to be determined.

In the present study we examine class II expression in double mutant mice lacking both Ii chain and DM functions. For the most part, their cellular and biochemical defects closely parallel those observed for Ii chain mutants. Thus we found that Ii−DM− double mutants display markedly reduced class II surface expression, peptide occupancy, and CD4+ T cell development. The loss of DM function has no noticeable effect on the production of floppy

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5 Abbreviations used in this paper: Ii, invariant; ER, endoplasmic reticulum; CLIP, class II-associated Ii chain peptide; PE, phycoerythrin.

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Aαbββ dimers. Additionally, our experiments demonstrate that expression of floppy Aαbββ dimers is TAP independent. Compared with Ii chain mutants, li DMβ double-mutant spleen cells consistently display increased peptide binding activities and enhanced presentation abilities in T cell stimulation assays. This probably reflects higher levels of Aαbββ surface molecules associated with an increased representation of B lymphocytes. B cell maturation is partially defective in the absence of li chain or DM expression and, interestingly, appears more severely compromised in li DMβ double mutants. These mutant mice lacking both li chain and DM functions should prove useful for studying class II peptide acquisition in the intact animal.

Materials and Methods

Mice

TAP-1 mutant mice (29), Ii chain mutants (23), DMβ-deficient mice (26), and C57BL/6 mice carrying a targeted disruption at the Aα locus (30) were previously described, and have been maintained by brother-sister matings. To generate double mutants, we set up intercross matings between heterozygous F1 progeny. Genotyping for the TAP-1 mutant allele was performed by Southern blotting of tail DNA as previously described (29). PCR genotyping assay used to distinguish wild-type and li chain mutants has been described (3). Additionally, to screen for the DMβ mutant allele, we used a three-primer system. The common primer (5′-TCTGGACACTGGATTGACCTC-3′) lying at the 3′ end of exon 2 used in conjunction with a second primer upstream (5′-CACATTCCCCGACACTCTATCTG-3′) in a portion of the gene deleted by the targeting event yields a 270-bp wild-type band. Additionally, a third primer (5′-ATCGCCCTCATGCGCTCTTCAC-3′) specific for the neo cassette in the targeting vector gives rise to the 362-bp mutant product. Reactions were conducted (38), given to us by Sasha Rudensky (University of Washington, Seattle, WA). IL-2 production was assessed by incubating T cells (5 × 10^5/well) with irradiated (3300 rad) spleen cells (2 × 10^5/well) in 200 µl of complete RPMI 1640 supplemented with 15% FCS, 10% NCTC109, 100 U/ml penicillin, 100 µg/ml streptomycin, 1 mM sodium pyruvate, 15 mM HEPES (pH 7.2), 0.1 mM nonessential amino acids, 5 × 10^{-3} M 2-ME, 2 mM glutamine, and increasing concentrations of Ag as indicated in Figure 4. Supernatants were collected after 20 h and assayed for IL-2 content in a secondary culture using CTL indicator cells. 3H]hypoxanthine incorporation was measured in the presence of 50% primary supernatant. Responses were measured after a 48-h culture by a 16- to 18-h exposure to 1 µCi of [3H]hypoxanthine. All results are expressed as mean counts per minute of triplicate cultures.

Results

DM- and TAP-independent expression of floppy Aαbββ dimers

Recent experiments suggest that DM associates with empty class II molecules to prevent their irreversible misfolding or aggregation after CLIP release (16–18). A strong argument can be made that floppy Aαbββ conformers produced by li chain mutants lack peptide ligand(s) (3, 23). It was therefore of interest to test the possibility that DM activity may be necessary for expression of floppy Aαbββ dimers. To this end, we set up matings between li chain and DM mutant strains. Their doubly heterozygous offspring were subsequently intercrossed to produce homozygous li DMβ mutants. Additionally, to examine possible contributions made by the class I peptide loading pathway, we generated TAP- li doubly deficient animals. We then compared these double-mutant spleen cells for their expression of floppy Aαbββ dimers in immunoprecipitation experiments.

As shown in Figure 1 after 5 h of chase, virtually all mature AαAββ molecules produced by wild-type splenocytes migrated as compact dimers at approximately 56 kDa, whereas, in contrast, floppy Aαbββ dimers produced by li chain mutants displayed reduced mobilities on SDS-PAGE. Consistent with previous results, in the nonboiled samples we found abundant AαAββ/CLIP complexes migrating at an intermediate position expressed by DM mutant spleen cells. In comparison with li chain mutants, roughly equal amounts of floppy Aαbββ dimers were produced by both li DMβ and li TAPβ double-mutant spleen cells. Moreover, these floppy Aαbββ conformers displayed equivalent reactivities with both Y3P (α+β) and M5/114 (β-specific) mAbs directed against conformational determinants. Thus, expression of floppy Aαbββ dimers is independent of both DM and TAP functions.
Next, we examined Ii<sup>2</sup>DM<sup>2</sup> double mutants for their class II surface expression. As judged by staining with Y3P (α + β) mAb, Ii<sup>2</sup>DM<sup>2</sup> splenocytes expressed reduced amounts of total surface A<sub>a</sub>A<sub>b</sub>A<sub>b</sub> roughly equal to Ii chain mutants (Fig. 2). As expected, we found that DM mutant spleen cells express 30-2 epitopes, whereas Ii<sup>2</sup>DM<sup>2</sup> double-mutant spleen cells fail to react with 30-2 mAb specific for surface A<sub>a</sub>A<sub>b</sub>A<sub>b</sub>/CLIP complexes (33). In contrast with diverse I-A<sub>b</sub>/peptide complexes expressed on the surface of wild-type spleen cells, the I-A<sub>b</sub>/CLIP complexes produced by DM mutants showed no reactivity toward BP107 (β-specific) mAb (27, 28). Consistent with earlier results, we observed that A<sub>a</sub>A<sub>b</sub>A<sub>b</sub> surface molecules expressed by DM mutant spleen cells selectively lacked BP107 epitopes. Surprisingly, in contrast, we found that Ii<sup>2</sup>DM<sup>2</sup> double-mutant splenocytes gained reactivity with BP107 mAb. Thus, loss of DM function appears to promote surface display of selected A<sub>b</sub>A<sub>b</sub> conformational determinants, possibly due to binding site occupancy by a specific peptide ligand(s).

**FIGURE 1.** DM- and TAP-independent production of floppy A<sub>a</sub>A<sub>b</sub>A<sub>b</sub> dimers. Cytoplasmic extracts prepared from spleen cells labeled with [<sup>35</sup>S]methionine for 40 min and chased for 5 h were immunoprecipitated with the indicated mAbs. Complexes solubilized at 100°C (B) or at room temperature (NB) were analyzed on 10% polyacrylamide gels under reducing conditions. C and F indicate the positions of compact and floppy dimers, respectively. Under these conditions, the CLIP peptide migrates just ahead of the dye front.

**FIGURE 2.** A<sub>a</sub>A<sub>b</sub>A<sub>b</sub> surface expression. Splenocytes from +/- +/- (1), DM<sup>+</sup> +/- (2), +/- li<sup>-</sup> (3), or DM<sup>-</sup> li<sup>-</sup> (4) mice were stained with biotin-conjugated mAbs followed by FITC-conjugated avidin.

Ii<sup>-</sup>DM<sup>-</sup> spleen cells exhibit increased functional activities

To test Ii<sup>-</sup>DM<sup>-</sup> double mutants for their peptide binding capabilities, we used Y-Ae mAb reactive with A<sub>a</sub>A<sub>b</sub>/E<sub>a</sub>56–73 complexes (32). Splenocytes were cultured with E<sub>a</sub>56–73 peptide and then analyzed in surface staining experiments. As expected, DM mutant spleen cells displayed severely compromised peptide loading abilities, reflecting their expression of stable A<sub>a</sub>A<sub>b</sub>/CLIP complexes (Fig. 3). We also observed markedly increased amounts of E<sub>a</sub>56–73 peptide bound by Ii chain mutant spleen cells. The Ii<sup>-</sup>DM<sup>-</sup> double mutants consistently displayed increased percentages of Y-Ae-positive cells, unaccompanied by an upward shift in fluorescence intensity. Surface staining with Y3P mAb was increased to the same degree, suggestive of changes affecting representation of B lymphocytes. As shown in Figure 3 (Expt. 1), peptide acquisition by TAP<sup>+</sup> Ii<sup>-</sup> double mutants closely paralleled that observed for Ii-deficient mice.

Next, we analyzed Ag presentation abilities in T cell stimulation assays. As expected, DM mutant spleen cells appeared relatively ineffective for presentation of already processed peptides or intact protein Ags (Fig. 4). Ii chain mutant spleen cells were defective for presentation of native protein Ags and displayed markedly enhanced abilities to present already processed peptides. We found

Next, we analyzed li<sup>-</sup>DM<sup>-</sup> double mutants for their class II surface expression. As judged by staining with Y3P (α + β) mAb, li<sup>-</sup>DM<sup>-</sup> splenocytes expressed reduced amounts of total surface A<sub>a</sub>A<sub>b</sub>A<sub>b</sub> roughly equal to li chain mutants (Fig. 2). As expected, we found that DM mutant spleen cells express 30-2 epitopes, whereas li<sup>-</sup> DM<sup>-</sup> double-mutant spleen cells fail to react with 30-2 mAb specific for surface A<sub>a</sub>A<sub>b</sub>A<sub>b</sub>/CLIP complexes (33). In contrast with diverse I-A<sub>b</sub>/peptide complexes expressed on the surface of wild-type spleen cells, the I-A<sub>b</sub>/CLIP complexes produced by DM mutants showed no reactivity toward BP107 (β-specific) mAb (27, 28). Consistent with earlier results, we observed that A<sub>a</sub>A<sub>b</sub>A<sub>b</sub> surface molecules expressed by DM mutant spleen cells selectively lacked BP107 epitopes. Surprisingly, in contrast, we found that li<sup>-</sup>DM<sup>-</sup> double-mutant splenocytes gained reactivity with BP107 mAb. Thus, loss of DM function appears to promote surface display of selected A<sub>b</sub>A<sub>b</sub> conformational determinants, possibly due to binding site occupancy by a specific peptide ligand(s).
that Ii<sup>-</sup>DM<sup>-</sup> spleen cells similarly lacked the ability to present intact protein Ags, and they consistently had marginally higher responses in the presence of already processed peptides. These enhanced functional activities potentially reflect increased percentages of B lymphocytes expressing class II surface Ags, as discussed below.

A recent study describes defective B cell maturation caused by the loss of Ii chain expression (39). The results of functional assays presented above suggest that Ii<sup>-</sup>DM<sup>-</sup> double mutants contain increased percentages of B lymphocytes. For these reasons, we decided to analyze B cell subpopulations present in our mutant strains. As a control, we also tested class II<sup>-</sup> mutant mice for their representation of B cell subsets. As a marker for mature B cells, we examined the expression of CD23, the low affinity IgE Fc receptor (40, 41). Spleen and lymph node IgM<sup>+</sup> B cells were also analyzed for their coexpression of surface IgD. As expected in wild-type cells, the predominantly mature IgM<sup>+</sup> B cells coexpressed both IgD and CD23 surface markers (Fig. 5). Consistent with previous results, we found here increased percentages of immature B cells lacking surface IgD and CD23 expression present in Ii chain mutants. As shown in Figure 5, DM mutants similarly displayed defective B cell maturation. The representation of immature B cells was markedly increased in Ii<sup>-</sup>DM<sup>-</sup> double mutants. Interestingly, we observed a striking reduction in the total number of IgM<sup>+</sup> B cells in lymph node populations from mutants lacking either Ii chain or class II expression. Similar conclusions were reached in double-staining experiments using CD45R/B220 as the pan-B cell marker (data not shown). These findings support the idea that Ii chain expression is necessary to promote B cell maturation. Moreover, our results strongly suggest that class II expression also contributes to B cell development.

**B cell development is severely compromised in Ii<sup>-</sup>DM<sup>-</sup> double mutants**

FIGURE 3. Peptide binding capabilities. Splenocytes from +/+ (1), DM<sup>-/+</sup> (2), +/+ Ii<sup>-</sup> (3), DM<sup>+</sup>Ii<sup>-</sup> (4), or TAP<sup>-</sup>Ii<sup>-</sup> (5) mice were cultured for 5 h at 37°C in the presence of Ea<sub>56–68</sub> and then stained with biotin-labeled mAbs followed by FITC-avidin. Control spleen cells cultured in medium alone gave no detectable Y-Ae staining above background.

FIGURE 4. Ag presentation activities. Cultures contained spleen cell populations (2 × 10<sup>5</sup>), I-A<sup>b</sup>-restricted T hybridomas (5 × 10<sup>4</sup>), and increasing concentrations of peptides or protein Ag, as indicated. Representative data from one of four identical experiments with similar results are shown.
Decreased CD4⁺ and CD8⁺ T cell maturation in the absence of DM expression

Mutants lacking either Ii chain (23–25) or DM (26–28) expression display partially defective CD4⁺ T cell maturation. It was therefore of interest to examine the extent of CD4⁺ T cell development in Ii⁻DM⁻ double mutants. Consistent with previous results we also observed that Ii chain and DM mutants contained reduced numbers of mature CD4⁺ T cells in the thymus and periphery (Fig. 6). Doubly deficient Ii⁻DM⁻ mutants similarly lacked mature CD4⁺ T cells, and their CD4⁺ percentages often appeared slightly reduced compared with those observed for Ii chain or DM mutants. Interestingly, Ii⁻DM⁻ double mutants did not contain increased percentages of peripheral CD8⁺ T cells as observed for Ii chain mutants. Thus, we conclude that Ii⁻DM⁻ double mutants exhibit decreased T cell maturation potentially due to their lack of surface AαBβ/CLIP complexes.

Discussion

The Ii chain is an essential class II chaperone, guiding selective peptide capture in the endocytic pathway (1, 2). In its absence, the vast majority of class II molecules fail to acquire endoglycosidase H-resistant glycans and are rapidly degraded; class II surface expression is markedly reduced, but is not completely eliminated (23–25). As for floppy conformers generated upon exposure of compact dimers to low pH in the absence of peptide (42), the few mature AαBβ molecules expressed by Ii chain mutant mice display reduced mobilities in SDS gels and by functional criteria appear to lack self peptide ligand(s) (3, 23, 25). To test whether production of floppy AαBβ dimers depends on DM or TAP functions, we examined class II structure in Ii⁻DM⁻ and TAP⁻Ii⁻ double mutants.

It is possible that truly empty AαBβ molecules escape ER quality control. On the other hand, considerable data suggest that binding site occupancy is essential for class II export through the secretory pathway (43–45). In the absence of an Ii chain, the empty groove of AαBβ dimers potentially associates with peptides available inside the ER lumen. According to this way of thinking, the production of floppy AαBβ conformers may depend on activities contributed by the class I peptide transporter (TAP). Recent studies demonstrate that TAP preferentially interacts with short peptides of approximately 8 to 10 residues, an appropriate length for class I binding (46). However, longer peptides up to 40 residues in length also function as TAP substrates.

![Figure 5](http://www.jimmunol.org/Downloadedfrom)
and are thus available for class II loading (47). To determine whether the expression of floppy \( \alpha \beta \) dimers depends on TAP activity, we generated TAP–Ii double-mutant mice. Similar TAP–Ii double mutants were recently described by Tourne et al. (48). They also found roughly equal amounts of mature \( \alpha \beta \) dimers produced by Ii chain mutants and double mutants lacking TAP function. Additionally, the present experiments demonstrate that these floppy \( \alpha \beta \) dimers are equally reactive with mAbs directed against distinct conformational epitopes contributed by both \( \alpha \)- and \( \beta \)-chains. Perhaps floppy \( \alpha \beta \) conformers devoid of peptide exit the ER and are transported to the cell surface. On the other hand, previous studies also describe class I loading of signal sequence-derived peptides (49, 50). Thus, TAP-independent expression of floppy \( \alpha \beta \) dimers potentially reflects transient association with signal peptides or perhaps intact ER polypeptides (51, 52).

Recent studies suggest that DM associates with empty class II molecules to prevent their irreversible misfolding or aggregation in endocytic compartments (16–18). The cytoplasmic tails of class II \( \beta \)-chains also contain an endosomal targeting signals(s) (53). Thus, generation of floppy \( \alpha \beta \) dimers could also be caused by peptide loss upon exposure to acidic pH inside the endocytic compartment(s) and thus be dependent on DM chaperone functions. The present experiments demonstrate that this is not the case. Roughly equal amounts of floppy \( \alpha \beta \) dimers were produced by Ii chain and Ii–DM double mutants. Thus, structurally distinct \( \alpha \beta \) dimers lacking tightly bound self peptide ligand are probably exported via the constitutive secretory route.

In functional experiments, Ii–DM double mutants consistently had slightly higher responses compared with mice lacking Ii chain alone. Similar results were obtained in both peptide binding and T cell stimulation assays. Thus, we observed higher percentages of \( Y-Ae \)-positive cells following incubation with \( E_{26-73} \) peptide, for the most part unaccompanied by an upward shift in peak fluorescence intensity. Because surface staining with \( Y3P \) mAb was increased to the same degree, it seemed likely that Ii–DM double mutants might simply contain increased percentages of B lymphocytes. To examine this possibility, we compared B cell representation in Ii chain, DM, and double mutant strains. As described in original reports (23–25), we also observed here for Ii chain-deficient splenocytes, only slightly reduced B cell percentages in surface staining experiments using IgM or CD45R/B220 as a pan-B cell marker. Recent experiments analyzing spleen, bone marrow, and Ag-primed lymph node cell populations suggest that Ii chain function is required for B cell maturation (39). It was therefore of interest to evaluate the extent of B cell development in our mutant mouse strains. Consistent with recent data, we also found here that Ii chain-deficient mice contain higher percentages of immature B cells. Interestingly, mutant mice lacking DM function also display defective B cell maturation, and Ii–DM double mutants exhibit a
more severe phenotype. In contrast to results obtained by analyzing splenocytes, we found that the total representation of IgM⁺ B cells was markedly reduced in lymph nodes from mutants lacking LI chain or class II expression.

The possible relationship(s) between B cell development and class II surface expression has been intensely investigated. Surface class II appears late during B cell development coincident with the onset of surface IgD expression (54) and is up-regulated as a consequence of cross-linking Ig surface receptors (55). Ag uptake via surface Ig facilitates class II Ag presentation (56, 57). Interestingly, class II expression distinguishes B cell developmental pathways during ontogeny (58, 59). However, experiments to date collectively argue that B cell development is independent of class II surface expression. For example, there is general agreement that class II mutant mice exhibit a normal Ab response to T-independent Ags (30, 60–62). The lack of responses directed toward T-dependent Ags and the absence of germinal centers in class II-deficient mouse strains have been attributed to the near-complete elimination of mature CD4⁺ T cells (30, 60–62). Initial observations suggested that these animals lack mature IgM⁺ IgD⁺ B cells (60), but subsequent reports demonstrate normal B cell development in class II mutant strains (39, 62). In contrast, recent experiments suggest that LI chain function is necessary for B cell maturation and production of T-independent Abs (39).

The present experiments demonstrate that LI chain, DM, and class II mutant mice all exhibit B cell defects. It is possible that such discrepancies reflect strain differences, because these targeted mutations were independently established on a mixed (129 × C57BL/6)F2 genetic background and have been separately maintained in different laboratories. Moreover, the present study describes defective B cell maturation caused by targeted disruption of the Aoβ gene (30); in contrast, other investigators analyzed class II-deficient mice created by targeting the Aαβ dimers (39). Perhaps normal B cell development observed in these mice reflects their expression of mixed Aoαβ heterodimers. These contradictory results may also reflect variable animal health status in different labs. The underlying mechanism for defective B cell maturation remains unclear. Adoptive transfer experiments reported by Shachar and Flavell (39) are consistent with an intrinsic block to B cell development. Thus, B cell survival in the competitive follicular environment may be compromised due to the loss of intracellular signaling via surface class II. Alternatively, class II surface display of diverse peptides may be necessary for thymic development and activation of mature CD4⁺ helper T lymphocytes producing inducible cytokines. Additional experiments are needed to distinguish between these possibilities.

Numerous reports describe LI chain-independent presentation of selected T cell epitopes (24, 53, 63–69). Similarly, previous studies document class II peptide loading via an alternative DM-independent pathway (67–71). Self peptides available within the constitutive secretory pathway may associate with empty class II molecules inside the ER (45, 72, 73), during transport through the Golgi, or at the cell surface. Additionally, mature recycling class II molecules can present selected epitopes in an LI chain- and DM-independent manner (53, 67, 68). On the other hand, it is also known that association with the LI chain prevents class II peptide occupancy (13, 74–76) and blocks presentation of selected epitopes (66). Similarly, DM facilitates Ag presentation via the conventional pathway and inhibits presentation of a significant fraction of endogenous self peptides (77, 78). Consistent with these findings, we observed that DM mutants fail to express BP107 epitopes, but LI⁻DM⁻ double mutants display BP107 reactivity. These results strengthen the idea that LI chain and DM both positively and negatively influence class II surface display of self peptide ligands by an as yet poorly understood mechanism(s).

Previous studies characterizing LI chain and DM functional activities have extensively used established cell lines. The relative expression levels of class II, LI chain, and DM are clearly an important factor determining the outcome of these experiments. Moreover, transfection recipients used in functional experiments may differ in their content of organelles, proteases, and molecular chaperones, and recent experiments demonstrate that these cell type-specific differences have a significant impact on the intracellular distribution of class II molecules (79). Particularly in the case of heterologous expression systems comprised of introducing murine constructs into human mutant cell lines and vice versa, subtle structural differences affecting transient associations among class II αβ dimers, LI chain, and DM are likely to have a significant impact on the diverse array of self peptides presented at the cell surface. Recent experiments also demonstrate the important influence contributed by endogenous peptides (77, 78). Mutant spleen cell populations described in this report are identical in every respect, except for their DM and LI chain expression. Nonetheless, their surface display of BP107 epitopes exhibits a complex pattern of regulation.

MHC class II, LI chain, and DM genes show similar tissue-specific patterns of expression and are coordinately up-regulated in response to cytokines. However, there is also evidence for non-co-ordinate expression of class II and LI chain in selected cell types (80–83). It seems likely that subtle imbalances affecting the relative levels of class II, LI chain, and DM expression may influence the presentation of self peptides by nonprofessional accessory cells, such as thyroid follicular cells, astrocytes, and pancreatic β cells under pathologic conditions, and potentially results in exposure of neopeptides that initiate autoimmune responses. Mutant mice selectively lacking LI chain and DM activities should prove useful for analyzing nonconventional class II Ag presentation by diverse types of accessory cells in the intact animal.

Note added in proof. While this manuscript was under review, a similar study analyzing mice doubly deficient for LI chain and H-2M complexes was published by Tourne et al. (84).

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